Provirus Load in Breast Milk and Risk of Mother-to-Child Transmission of Human T Lymphotropic Virus Type I

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In a prospective study of 101 mother-child pairs in Jamaica, we examined the association of provirus load in breast milk and the risk of mother-to-child transmission of human T lymphotropic virus type I. The provirus load in breast milk was a strong predictor of risk of transmission to children (relative risk, 2.34/quartile), after adjustment for other known risk factors. The risk of transmission increased from 4.7/1000 person-months when the provirus load in breast milk was <0.18% to 28.7/1000 person-months when it was >1.5%. Provirus detection in maternal breast milk predicted transmission months before infection in children was detected by serologic testing.

Human T lymphotropic virus type I (HTLV-I) is etiologically associated with adult T cell leukemia/lymphoma (ATL), but <5% of HTLV-I–infected persons develop ATL [1]. Transmission of this virus occurs from mother to child [2, 3], by sexual contact [4], or by the transfusion of cellular blood components [5, 6]. Vertical transmission is an important route of transmission, because infection early in life is associated with a subsequent risk of ATL [7].

Children born to HTLV-I–infected mothers acquire infection predominantly from breast-feeding [3, 7]. A longer duration of breast-feeding has been associated with an increased risk of infection [3, 8]. We have shown that the maternal HTLV-I provirus load in peripheral blood mononuclear cells (PBMCs) is an independent predictor of mother-to-child transmission [9]. We hypothesized that provirus load in peripheral blood is likely to be an indirect marker and that the provirus load in breast milk would be a more critical determinant of the risk of transmission. Thus, we examined the HTLV-I provirus load in breast milk and the risk of infection in children in a prospective study in Jamaica.

Subjects and methods. Subjects of the analysis were participants in the Jamaica Mother-Infant Cohort Study [3, 9]. The study enrolled 339 pregnant women (212 HTLV-I positive and 127 HTLV-I negative) attending antenatal clinics in Kingston between January 1989 and August 1990, with follow-up of their children extending over a decade. Samples of mothers' peripheral blood were collected at the time of delivery. Breast-milk samples were collected by use of a breast pump during a postnatal clinic visit, on average 11 days (range, 5–47 days) after delivery. Blood was collected from children at birth, every 6 weeks for the first 6 months, every 3 months until 2 years of age, and every 6 months thereafter. Samples were frozen at −70°C in a central repository. Information on maternal weekly income and breast-feeding status was obtained by use of a standardized questionnaire. The analyses were performed on all 101 HTLV-I–positive mothers with available breast-milk samples and their 104 children, of whom 23 became HTLV-I–positive at an average of 14 months of age. The median durations of follow-up for HTLV-I–infected children and HTLV-I–negative children were 9.8 and 6.4 years, respectively ($P = .48$). Informed consent was obtained from all study participants. The study protocol followed the human experimentation guidelines of the US Department of Health and Human Services and was approved by the institutional review boards of the National Cancer Institute and the University of the West Indies.

HTLV-I positivity was determined by a whole-virus EIA (Dupont) and confirmed by Western blot analysis (Cambridge-Biotech). Infection in children was defined as repeated HTLV-I positivity after 12 months of age. Determination of age at the time of infection used the midpoint between ages at the last negative and the first positive specimen [3]. Antibody titer levels were assessed by 5-fold end-point dilution method by use of an EIA (Genetic Systems or Cambridge-Biotech). Cells from breast milk were prepared by centrifugation at 10,000 *g* at room temperature for 5 min, followed by a wash with 0.5 mL of PBS (GIBCO BRL), and then were resuspended in 300 μL of cell-lysis solution and incubated for 60 min at 55°C with 1.5 μL of proteinase K (20 mg/mL; Gentra Systems). DNA was extracted

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Table 1. Human T lymphotropic virus (HTLV) type I provirus load among 101 HTLV-I–positive mothers in Jamaica, by children's HTLV-I status.

Maternal factor	All mothers (n = 101)	Mothers of HTLV-I–positive children (n = 23)	Mothers of HTLV-I–negative children (n = 78)
Provirus load in peripheral blood, %			
Undetected	19 (18.8)	2 (8.7)	17 (21.8)
<0.36	21 (20.8)	1 (4.3)	20 (25.6)
0.36–2.00	18 (17.8)	3 (13.0)	15 (19.2)
2.00–8.00	22 (21.8)	7 (30.5)	15 (19.2)
>8.00	21 (20.8)	10 (43.5)	11 (14.1)
Provirus load in breast milk, %			
Undetected	27 (26.7)	1 (4.3)	26 (33.3)
<0.18	18 (17.8)	2 (8.7)	16 (20.5)
0.18–0.60	19 (18.8)	3 (13.0)	16 (20.5)
0.60–1.50	20 (19.8)	8 (34.8)	12 (15.4)
>1.50	17 (16.8)	9 (39.1)	8 (10.3)
Provirus detection in breast milk	74 (73.2)	22 (95.7)	52 (66.7)

NOTE. Data are no. (%). Quartiles were set based on individuals with detectable levels of provirus load in each compartment. There were 19 and 27 persons who did not have an HTLV-I provirus load detected in peripheral blood and breast milk, respectively. P values were estimated from χ^2 statistics or Fisher's exact tests (when expected values in ⩾1 cells were <5), comparing the distribution of a variable between mothers of HTLV-I–positive and –negative children. $P = .004$, for differences in provirus load in peripheral blood; $P = .01$, for provirus load and detection in breast milk.

from 2×10^6 PBMCs and from 500 μL of breast milk, by use of the PureGene DNA Purification Kit (Gentra Systems).

Provirus load was measured by a real-time quantitative polymerase chain reaction (PCR) method by use of an ABI PRISM 7700 Sequence Detection System, as described elsewhere [10]. The provirus load in peripheral blood and breast milk are described as a percentage—that is, the number of copies detected per 100 cells. The sensitivity of detection was 0.01%.

The association of provirus detection and level in breast milk and the child's HTLV-I status was examined by χ^2 or Fisher's exact test. HTLV-I provirus loads in breast milk and peripheral blood were compared between mothers of HTLV-I–positive and –negative children, stratified by the duration of breast-feeding (>12 vs. ⩽12 months), by use of the Kruskal-Wallis test. Because distributions of provirus load in breast milk and peripheral blood were skewed and not linearly associated, correlations between these 2 variables were assessed by Spearman's correlation coefficient. Risks of HTLV-I infection among children with detection and level of maternal provirus were estimated by the relative risks (RRs) and their confidence intervals (CIs) derived from the proportional hazard models. Variables were treated as both continuous and categorical. When variables were categorical, the duration of breast-feeding (⩽6, 6–12, and >12 months) and maternal weekly income (⩽100, 101–200, and >200 Jamaican dollars [J$]) were in tertiles, HTLV-I titers were in quartiles, and HTLV-I provirus loads were categorized into 5 groups: undetected and at the quartiles among persons with detected levels [3, 9]. All statistical tests were 2-sided. The incidence of HTLV-I infection was calculated by the total number of new infections in children divided by the total person-months of follow-up.

Results. Provirus loads of 101 mothers, according to the child's HTLV-I status, are presented in table 1. Eighty-two (81.1%) and 74 (73.3%) of 101 mothers had provirus detected in the peripheral blood and breast milk, respectively. The median provirus load was 1.3% (range, 0.009%–80.0%) in peripheral blood, compared with 0.24% (range, 0.006%–19.1%) in breast milk. The absolute copy number of HTLV-I provirus ranged from 1 to >19,000 copies/mL breast milk, with a median of 10.8 copies/mL. The detection of provirus in breast milk was not associated with the interval between birth and the collection of samples ($P = .44$) or the duration of breast-feeding ($P = .36$). The correlation between provirus load in peripheral blood and that in breast milk was highly significant ($R = 0.57$; $P < .0001$).

Of the 104 children, 23 (22%) became infected. Twenty-two (95.7%) of 23 mothers of HTLV-I–positive children had provirus detected in breast milk, compared with 52 (66.7%) of 78 mothers of HTLV-I–negative children ($P = .01$; table 1). The provirus load in breast milk was significantly higher in mothers who transmitted HTLV-I to their children than in mothers who did not (median, 1.3% vs. 0.18%; $P < .0001$), irrespective of the duration of breast-feeding (figure 1*A*).

In unadjusted proportional-hazard analysis, the risk of HTLV-I transmission was associated with a higher provirus load in breast milk (RR = 1.79 per quartile [95% CI, 1.16–2.76]). The strength of association was similar to that for provirus load in peripheral blood (RR = 1.93 per quartile [95% CI, 1.28–2.92]). In multivariate analysis, the provirus load in breast milk re-

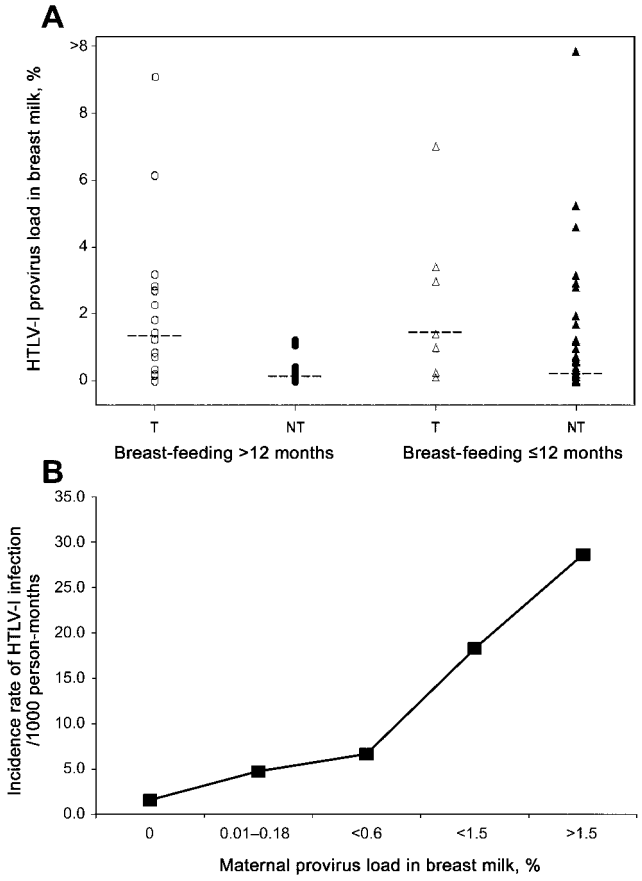

Figure 1. *A,* Distribution of provirus load (%) in breast milk in mothers who transmitted human T lymphotropic virus (HTLV) type I (Ts) and those who did not (NTs), by duration of breast-feeding (>12 vs. ≤12 months). Data were available for 44 long-term breast-feeders (16 Ts and 28 NTs) and 57 short-term breast-feeders (7 Ts and 50 NTs). The median provirus load *(broken lines)* was 1.3% in Ts and 0.13% in NTs among mothers who breast-fed for >12 months ($P = .0001$) and 1.4% in Ts and 0.21% in NTs among mothers who breast-fed for ≤12 months ($P = .02$). *B,* Incidence rate of mother-to-child transmission of HTLV-I by provirus load (%) in breast milk.

mained a strong predictor of HTLV-I transmission to children (RR = 1.98 per quartile [95% CI, 1.21–3.23]), after adjustment for antibody titer, duration of breast-feeding, and maternal income, all as categorical variables. Adjusting for the provirus load in breast milk, antibody titer was not a significant determinant of the risk of transmission (RR = 1.05 per quartile [95% CI, 0.62–1.80]). After additional adjustment for the provirus load in peripheral blood, the provirus load in breast milk remained the strongest viral marker of the risk of transmission (RR = 2.34 per quartile [95% CI, 1.37–4.01]). In this model, a provirus load in peripheral blood (RR = 1.88 per quartile [95% CI, 1.05–3.38]), lower maternal income (RR = 2.72 per tertile [95% CI, 1.34–5.50]), and longer duration of breast-feeding (RR = 2.72 per tertile [95% CI, 1.16–6.39]) remained independent risk factors for the transmission of HTLV-I. Multivariate models that included all variables on a continuous scale yielded similar results, with the provirus load in breast milk being a strong predictor of risk of transmission (RR = 2.38 per $\log_{10}$ increase in provirus load [95% CI, 1.09–5.22]). The incidence of infection increased with provirus load in breast milk, increasing from 4.7/1000 person-months when mothers had a provirus load of <0.18% to 28.7/1000 person-months when mothers had a provirus load >1.5% (figure 1*B*).

Discussion. Breast milk is known to be the primary vehicle for the vertical transmission of HTLV-I [11], but, to our knowledge, a quantitative assessment of the risks of transmission in relation to the provirus load in breast milk has not been done previously. In the present study, we detected HTLV-I provirus in 73% of breast-milk samples from 101 seropositive women. In another study, provirus was detected by PCR in the breast milk of 8 (89%) of 9 seropositive Japanese mothers [12].

A high correlation between provirus load in peripheral blood and that in breast milk was not unexpected and would probably explain the previously reported association between transmission and provirus loads in peripheral blood [9]. However, the provirus loads in breast milk were lower than those in peripheral blood. This is likely because the majority of cells in breast milk are not lymphocytes, whereas provirus loads in peripheral blood were measured in PBMCs. One study reported that, among the lymphocyte subpopulations, there were similar proportions of HTLV-I–positive cells in peripheral blood and breast milk [13].

We examined provirus loads per 100 cells and found that the risk of HTLV-I transmission increased with both the detection and level of provirus in breast milk. The observation of higher risk of HTLV-I transmission among children born to mothers with provirus detected in breast milk is similar to observations for HIV [14]. However, transmissions of HTLV-I occurred in children exposed to breast milk with little or no provirus detected in the sample, which suggests that the number of cells in breast milk may vary over the duration of lactation in the same woman [15]. Because breast-feeding often continues for several months or longer, infants could have considerable exposure, even when the milk contains very low levels of provirus. We were unable to investigate this possibility because neither the amount of breast milk consumed, the variability in cellular composition in breast-milk samples, nor serial breast-milk samples were available.

In unadjusted analysis, the risk of transmission increased by 1.79- and 1.93-fold per quartile of provirus load in breast milk and peripheral blood, respectively. However, other factors that might increase or reduce the exposure of infants to HTLV-I in breast milk—such as the elevated lymphocyte levels in milk due to mastitis or differences in weaning practices—might have also confounded the association. In multivariate models that mutually adjusted for other variables that we could measure,

the risk of transmission was nearly 12.8-fold higher among mothers in the highest quartile of provirus load in breast milk, compared with those in the lowest quartile (2.34-fold/quartile).

Previously, we found that a high maternal HTLV-I antibody titer was an independent risk factor for mother-to-child transmission [3, 9]. In the present analysis, the provirus load in both breast milk and peripheral blood independently increased the risk of transmission, but antibody titer adjusted for provirus load did not. Thus, the reported association between antibody titer and the risk of transmission may have occurred because of residual confounding due to the strong correlation between the antibody titer and provirus load in peripheral blood.

The present study has also quantified provirus load in breast milk in association with the absolute risk of transmission from mothers to children. In our series, 22 of 23 transmissions occurred in children breast-fed by mothers who had provirus detected in their breast milk. However, of these 23 transmissions, 3 occurred in children exposed to breast milk that contained relatively low provirus loads (<0.18%), which suggests that low loads cannot be assumed to be innocuous and that most HTLV-I–infected mothers who breast-feed risk transmitting the virus to their children.

In summary, our data show the importance of provirus load in breast milk as a predictor of HTLV-I transmission from mothers to children. These results are consistent with breast milk being the primary vehicle of HTLV-I transmission. High antibody titers also predict the risk of infection well, largely because they are strongly correlated with the provirus load. In some endemic areas where the quantitation of provirus load is not readily available, the use of antibody titers might be a reasonable alternative for assessing the risk of mother-to-child transmission. The provirus load in blood remained an independent predictor of transmission in the multivariate model, which suggests that other mechanisms also may have contributed to the risk of infection.

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